GBclonart Seamless Assembly Kit

The GBclonart Seamless Assembly Kit provides a convenience, fast, high-throughput cloning of DNA fragment and generation of precise, directional constructs with any vectors that are ready for any purpose. The Seamless cloning technology allows cloning your gene or sequence of interest into any vector in one day without the need for restriction enzymes, ligase, or blunt-end polishing. The homologous recombination can occur in any site in vector only if the tail of fragment has 15-25 bp homologous to the corresponding fragment or vector.

Number	Name	specification	volume	storage
GB2002-12	GBclonart Seamless Assembly Kit	12 recations	180ul	-20
GB2002-24	GBclonart Seamless Assembly Kit	24 recations	360ul	-20
GB2002-48	GBclonart Seamless Assembly Kit	28 recations	720ul	-20

Storage: store at -20 ^{0}C, the validity period is a year, avoid being repeated freezing and thawing.

Product Feature:

I. flexible cloning sites: any sites in any vector.

II. fast and convenient: construct the vector in 30 minutes.

III. accuracy: no extra steps.

IV. efficient: 90% positive clone.

Steps:

I. Liner Vector Preparation

1. Restriction Enzymes:
Using appropriate enzyme to digest the vector. Double enzymes digestion is strongly recommend rather than single in order to improve the rate of positive clone. Purify the vector through DNA gel electrophoresis.

2. PCR:

Design forward and reverse primers in appropriate site. Using high fidelity DNA polymerase to get better results. Purify the vector through DNA gel electrophoresis to get rid of plasmid template and improve the rate of positive clone.

II. Fragment Preparation

15-25bp sequences are required in both ends of fragment, which asks a special pair of primers. The sequences should be homologous to the liner vector. As for the primer, each primer should be at least 35-40bp, including 15bp homologous sequences and 20-25 specific sequences.

Note: Check the reading frame when designing primers which should be purified by HPLC or PAGE. High fidelity DNA polymerase is also needed.

III. Recombination Reaction

1.Mix fragment and vector in appropriate mole ratio in EP tube to reaction. 50-100 ng vector is needed.

reaction system:

2X GBclonart recombination liquid:	10ul
liner vector:	X ul
PCR fragment A:	X ul
H_2O:	X ul
total:	20ul

2. 45 ^{0}C water bath the EP tube at least 30minutes before put it on ice.

3. Transform the mixture into competence cell.

Note:Fragment:Vector=2:1~3:1, 30 minutes is the best reaction condition.

IV. Transformation

1. Add 5ul mixture into 100ul competence cell, keep it on ice at least 5minutes

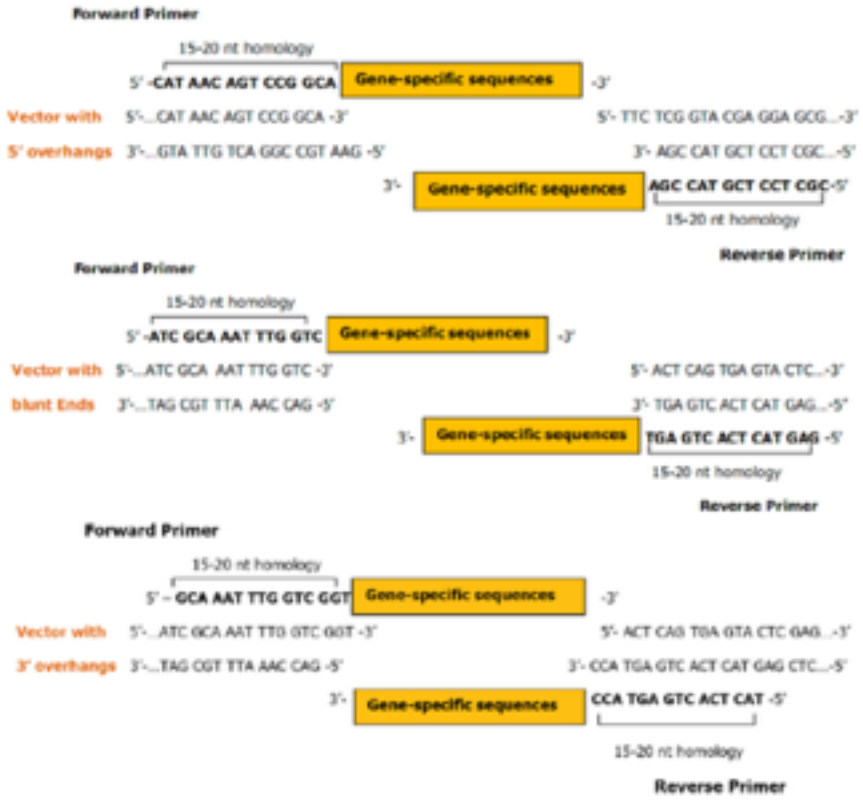
2. 42 ^{0}C water bath 1minutes before put it back on ice at least 2 minutes

3. Add 400ul LB or SOC, 37 ^{0}C shake culture an hour

4. Coat 200ul LB on LB plate with specific antibiotic, 37 ^{0}C culture the whole night. Check the clone in next day.

Example of Primer Design

1. Liner Fragment Primer



2. Primer for Ligation of Nearby Fragment